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Telomerase activity in the regenerative basal layer of the epidermis in human skin and in immortal and carcinoma-derived skin keratinocytes

(human keratinocytes/cell immortalization/senescence)

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ABSTRACT **Cellular senescence is defined by the limited proliferative capacity of normal cultured cells. Immortal cells overcome this regulation and proliferate indefinitively. One step in the immortalization process may be reactivation of telomerase activity, a ribonucleoprotein complex, which, by *de novo* synthesized telomeric TTAGGG repeats, can prevent shortening of the telomeres. Here we show that immortal human skin keratinocytes, irrespective of whether they were immortalized by simian virus 40, human papillomavirus 16, or spontaneously, as well as cell lines established from human skin squamous cell carcinomas exhibit telomerase activity. Unexpectedly, four of nine samples of intact human skin also were telomerase positive. By dissecting the skin we could show that the dermis and cultured dermal fibroblasts were telomerase negative. The epidermis and cultured skin keratinocytes, however, reproducibly exhibited enzyme activity. By separating different cell layers of the epidermis this telomerase activity could be assigned to the proliferative basal cells. Thus, in addition to hematopoietic cells, the epidermis, another example of a permanently regenerating human tissue, provides a further exception of the hypothesis that all normal human somatic tissues are telomerase deficient. Instead, these data suggest that in addition to contributing to the permanent proliferation capacity of immortal and tumor-derived keratinocytes, telomerase activity may also play a similar role in the lifetime regenerative capacity of normal epidermis *in vivo*.**

Two recent findings have reinvigorated interest in the process of cell immortalization. (*i*) Fusion experiments of normal and immortal cells had shown that a limited lifespan in culture has a genetic origin and is dominant over immortality (1). (*ii*) A good correlation was found between immortal and tumor cells and high-level expression of telomerase activity, whereas most corresponding normal cells were telomerase negative (2–6). These data suggest that activation of telomerase activity might be correlated with the process of immortalization (for review see ref. 7).

Telomerase is a ribonucleoprotein complex, which by means of RNA template adds TTAGGG telomeric DNA repeats onto the ends of chromosomes, thereby preventing the shortening of the chromosome ends, which is believed to protect the replicative capacity of cells (8, 9). While germ-line cells express telomerase activity and maintain their telomere length, most normal somatic cells are deficient in telomerase activity (10, 11) and telomeres were found to be shortened during *in vitro* cultivation. So far, only peripheral, cord blood, and bone marrow leukocytes from normal donors have been shown to express low levels of enzyme activity (12–14), whereas all other normal tissues were telomerase negative (15). Because only a low level of activity was seen in these tissues, it was interpreted that only a subset of cells expressed enzyme activity. However, the identity of the positive cells and the biological significance of telomerase activity in these cells remains to be determined.

By measuring telomere length in human fibroblast cultures no correlation was established between telomere length and the age of the donor, but a good correlation could be drawn between the initial length of telomeres and the number of cell divisions obtained with the different cells (16). Thus, it was proposed that inactivation of telomerase activity in normal cells results in progressive shortening of the telomeres with each cell cycle and that telomere shortening provides an "internal clock" (the Hayflick phenomenon), the basis for senescence of normal somatic cells (3). If this is the case, telomerase activity would be useful in discriminating senescent from immortal cells.

To test this hypothesis, we compared the telomerase activity in immortalized human skin keratinocytes with that in cell lines established from human skin squamous cell carcinomas (SCCs). The skin is a combination of tissues. The epidermis that protects the body as an outer barrier rests upon the basement membrane that joins the epidermis to the connective tissue, also called dermis, and the underlying subcutaneous fat. The epidermis is one of the self-renewing tissues that remains proliferative throughout the human lifetime with a turnover of generally 26–42 days. Within the epidermis, proliferation only takes place in the basal layer. From there the keratinocytes migrate through several suprabasal layers and undergo a continuous process of differentiation terminated in the formation of dead horn squames (17). In contrast, the dermis is poor in cellular components and mostly consists of extracellular matrix components.

We found telomerase activity in the SCC cell lines and in the *in vitro* immortalized keratinocytes, and this was independent of the mode by which they were immortalized. Unexpectedly, we also found telomerase activity in the basal layer of the epidermis from normal human skin.

MATERIALS AND METHODS

Cells and Culture Conditions. All human *in vitro* immortalized and carcinoma cell lines used are listed in Table 1. The cells were cultivated as described earlier (18). Routine subcultures were obtained by disaggregating cells with 0.1% trypsin/EDTA solution and replating at a split ratio of 1:10. Accordingly, when the cells were subjected to the telomerase assay they were detached by the same trypsin/EDTA treatment, with the time of trypsinization depending on the cell type (fibroblasts approximately 5 min, normal keratinocytes or

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Abbreviations: SCC, squamous cell carcinoma; TRAP, telomeric repeat amplification protocol; SV40, simian virus 40; HPV 16, human papillomavirus 16.

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Table 1. Telomerase positive skin keratinocyte cell lines

Cell line	Mode of immortalization	Origin	Ref.
HaCaT	Spontaneous	*In vitro*	18
NM1	Spontaneous	*In vitro*	19
HaSV	SV40	*In vitro*	20
SVK14	SV40	*In vitro*	21
HPK IA	HPV 16	*In vitro*	22
HPK II	HPV 16	*In vitro*	23
SCL-I		Tumor derived	24
SCL-II		Tumor derived	25
SCC-12		Tumor derived	26
SCC-13		Tumor derived	26

SV40, simian virus 40; HPV 16, human papillomavirus.

cell lines up to 15 min). The trypsin reaction was stopped by adding serum containing medium.

Isolation of Normal Keratinocytes. Human skin samples were obtained from patients undergoing surgery. Only trunk skin and foreskin distant from tumors or pathological areas were used to determine telomerase activity. To separate dermis from epidermis, pieces of skin (approximately 50 mm^2) were incubated overnight at 4°C in 10 mM Hepes with 142 mM NaCl, 6.7 mM $MgCl_2$, 1.0 mM $CaCl_2$ (pH 7.4) containing 0.5 mg/ml thermolysin (Sigma) and the epidermis was peeled away from the dermis with sterile fine forceps as described by Jones and coworkers (27). To further disaggregate basal and differentiated keratinocytes, the epidermis was floated on 0.05% trypsin/0.025% EDTA solution for 2–15 min at 37°C. Single cell suspensions were obtained by vigorously pipetting the epidermal sheets. The cell numbers and size distribution were determined by using the CASY 1 cell counter (Schaerfe System, Reutlingen, Germany). The cells were aliquoted (10^5 cells), centrifuged, and stored as pellets at −80°C until assayed for telomerase activity.

TRAP Assay. Due to the limitations in human material we used the recently developed PCR based TRAP (Telomeric Repeat Amplification Protocol) assay instead of the conventional telomerase assay. This technique allows for detection of telomerase activity in a small number of cells or small amounts of tissue. The TRAP assay was performed as described (15). For preparation of telomerase extracts cells were detached from the culture dish by trypsin/EDTA treatment (see above), counted, and standardized to aliquots of 10^5 cells. A 10^5-cell aliquot was incubated on ice for 30 min in 200 μl of lysis buffer, or 50-100 mg of frozen tissue (skin, epidermis, or dermis) was homogenized in 200-250 μl of ice-cold lysis buffer using Eppendorf tubes and micropestles rotated by a drill at 450 rpm (Eppendorf, Hamburg, Germany). The tissue extract was centrifuged at 16,000 × *g* for 20 min at 4°C, and the supernatant rapidly frozen and stored at −80°C. Protein concentrations of extracts were determined using the BCA protein assay kit (Pierce). To disturb the RNA component of the telomerase (RNase treatment), 5 μl of extract were incubated with 1 μg of RNase Plus (5 Prime → 3 Prime/Boehringer Mannheim) for 20 min at 37°C. Enzyme activities were analyzed by serial dilution of the 10^5-cell aliquot to an equivalent of 1000, 100, and 10 cells for cell extracts and 6, 0.6, and 0.06 μg of protein for tissue extracts. Two μl of diluted extracts were assayed in 50 μl of reaction mixture (15). The PCR products were separated by electrophoresis on a 10% polyacrylamide gel and the 6-bp DNA ladders were visualized by autoradiography.

Indirect Immunofluorescence. Cryostat sections of intact skin, epidermal sheets, and dermal preparations (5–7 μm) were stored at −80°C before antibody reaction. After thawing, sections were rehydrated in PBS for 5 min and incubated overnight in a moist chamber at room temperature. As primary antibodies we used a rabbit antibody against mouse collagen type IV (dilution 1:100, Institute Pasteur, Lyon, France), which stained the basement membranes of the epidermal/dermal junction and blood vessels; an antibody, which stained the basal and suprabasal layers of the epidermis, kindly provided by Dennis Roop (Baylor College, Houston), and a mouse monoclonal antibody against the differentiation specific keratins K1 and 10 (K8/60, Renner, Dannstadt, Germany), which exclusively stained the suprabasal layers of the epidermis. Following three washes in PBS, sections were incubated with secondary antibodies (anti-rabbit or anti-mouse labeled with either FITC or Texas Red, all from Dianova, Hamburg, Germany) for an additional 45 min, washed in PBS, rinsed in 10 mM Tris·HCl buffer (pH 8.5), and covered with coverslips in Aqua mount (Lerner Laboratories, New Haven, CT). The staining was viewed under a microscope equipped with epifluorescence optics (model DMRD 35; Leitz). Tissue sections were also stained with hematoxylin/eosin for routine histology.

RESULTS

Telomerase Activity in Immortal and Tumorigenic Keratinocytes. If telomerase activity is fundamental for the ability of cells to permanently replicate in culture, immortal skin keratinocytes should express enzyme activity irrespective of how immortalization was induced. To test this, we investigated human keratinocyte lines established following introduction of SV40, as well as HPV 16. In addition, we included two spontaneously immortalized keratinocyte lines, one established from adult skin (HaCaT), and the other from foreskin keratinocytes (NM1). Relative telomerase activity levels were determined by diluting the cell lysates and typical ladder formation was detected in 1000, 100, and 10 cell equivalents as shown for the SV40 immortalized SVK14 cells, the HPV 16 immortalized HPK II cells, and the spontaneously immortalized HaCaT cells (Fig. 1). Thus, all lines, irrespective of the mode of immortalization, were telomerase positive (Table 1).

Since it was described for fibroblasts that telomerase activity was weak in early passages following immortalization and only

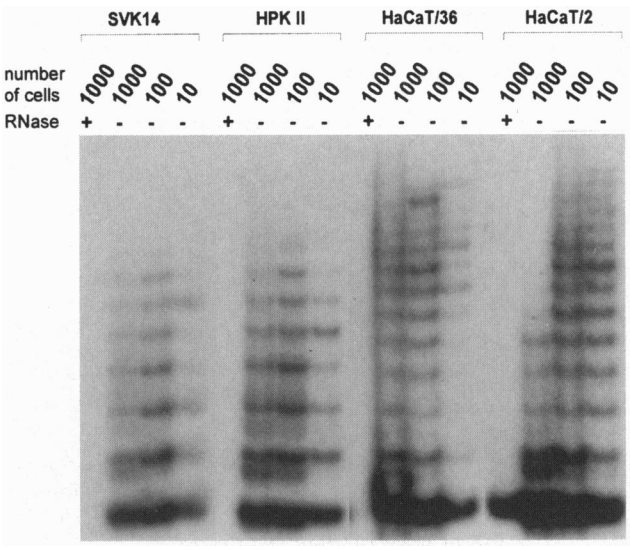

FIG. 1. Telomerase activity in *in vitro* immortalized human skin keratinocytes. SVK14 and HPK II are examples of cell lines immortalized by the DNA tumor viruses SV40 and HPV 16, respectively. The HaCaT cells represent one of the spontaneously immortalized lines. Extracts from 10^5 cells were subjected to the TRAP assay; reaction products were resolved on acrylamide gels and visualized by autoradiography. Telomerase activity was analyzed in 10-fold serial dilutions of the different cell extracts representing 1000, 100, and 10 cell equivalents. A 1000 cell aliquot was also treated with RNase as control. All cells and all passages of the spontaneously immortalized HaCaT cells (as demonstrated here for passages 36 and 2) showed a comparable telomerase activity.

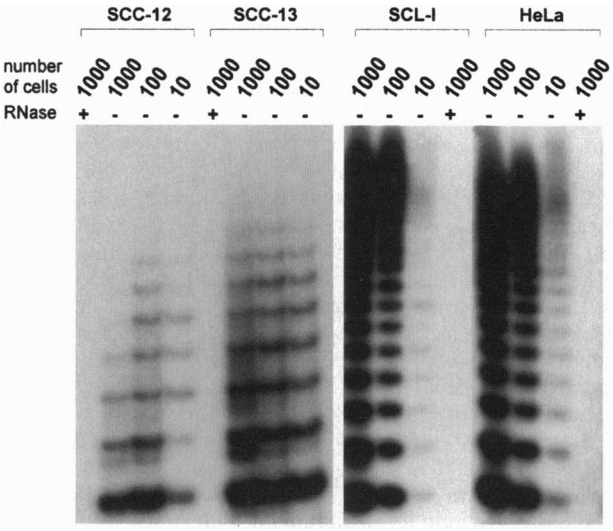

FIG. 2. Telomerase activity determined in 1000, 100, and 10 cell equivalents of SCC-12, SCC-13, and SCL-I cell lines established from SCCs of the skin. HeLa cells were included as a positive control. In all cell lines, ladder formation was detected in 1000, 100 and 10 cell equivalents.

increased with subsequent passages (28), we extracted cells from different passages of our spontaneously immortalized HaCaT cell line. We compared cells from passage 2, when the growth potential still largely resembled that of normal keratinocytes, with cells of passages 5, 7, 19, and 36 when they had gradually adapted to culture conditions and were finally able to either grow at clonal density or to form colonies in soft agar (18). In each case ladder formation was reproducibly detected in 1000, 100, and 10 cell equivalents, as shown for HaCaT cells of passage 36 and 2 in Fig. 1, suggesting that by passage 2, HaCaT cells expressed telomerase activity and that this activity was similar to that seen in later passage cells.

In contrast to the relative ease in immortalizing human skin keratinocytes *in vitro* by using oncogenic viruses, it still remains difficult to establish cell lines from skin carcinomas. So far only a few cell lines have been described, and they are all derived from human SCC, whereas no line has been established from a basal cell carcinoma, a nonmetastatic type of skin cancer. Therefore, we included only SCC lines in our study: SCC-12, SCC-13, SCL-I, and SCL-II. We detected telomerase activity in up to 10 cell equivalents in all cell lines, and enzyme activity in these cells was comparable to that of HeLa cells, used as a positive control (Fig. 2 and Table 1).

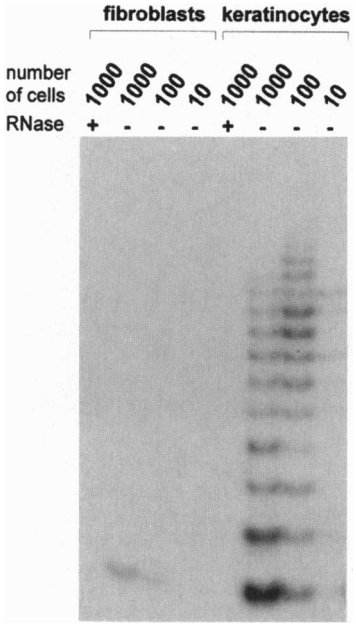

FIG. 3. Telomerase activity was determined in 1000, 100, and 10 cell equivalents of normal human skin fibroblasts and keratinocytes. Fibroblasts always remained telomerase negative, whereas keratinocytes showed enzyme activity in 1000 and 100 cell equivalents.

Telomerase Activity in Normal Human Skin. As controls, we also determined the telomerase activity in isolated normal dermal fibroblasts and epidermal keratinocytes. All extracts from fibroblasts, irrespective of passage number, were negative. However, unexpectedly, 14 different cultured primary and passaged skin keratinocytes, partly isolated from the same skin as the fibroblasts, exhibited enzyme activity (Fig. 3), with

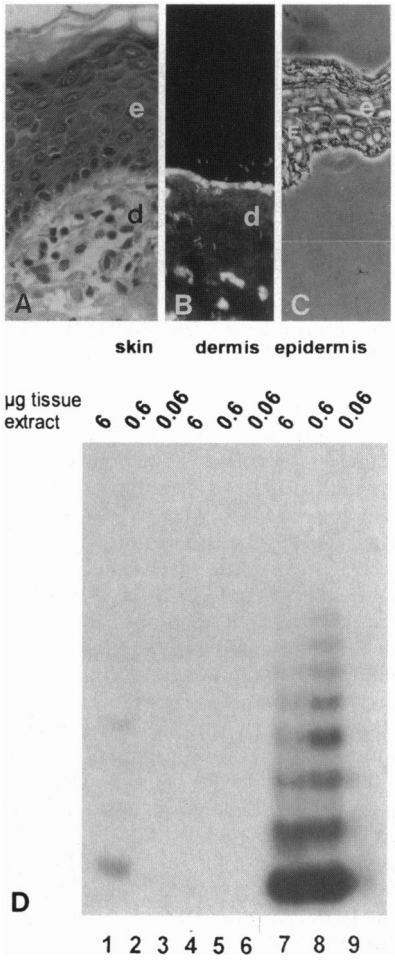

FIG. 4. Morphology and telomerase activity of normal human skin. (*A*) Histological section of the skin consisting of the multilayered epidermis (e) and the matrix-rich dermis (d) stained with hematoxylin/eosin. (*B*) The dermis was separated from the epidermis by thermolysin treatment and was labeled with an antibody against collagen type IV. Note that the immunofluorescent label shows that the basement membrane of the epidermal/dermal junction stayed with the dermis after separation. Immunofluorescent label also outlines the basement membrane around blood vessels. (*C*) Phase contrast micrograph of the isolated multilayered epidermis. (*D*) Telomerase activity determined in tissue extracts from one sample each of complete human skin, thermolysin separated dermis, and epidermis. For each tissue, samples of 6, 0.6, and 0.06 µg protein were analyzed. The complete skin specimen showed telomerase activity in the 6-µg tissue extract (lane 1), whereas it was negative at protein concentrations of 0.6 and 0.06 µg (lanes 2 and 3). The dermal lysate was telomerase deficient (lanes 4–6), whereas this epidermal sample showed ladder formation at protein concentrations of 6 and 0.6 µg but not at 0.06 µg (lanes 7–9).

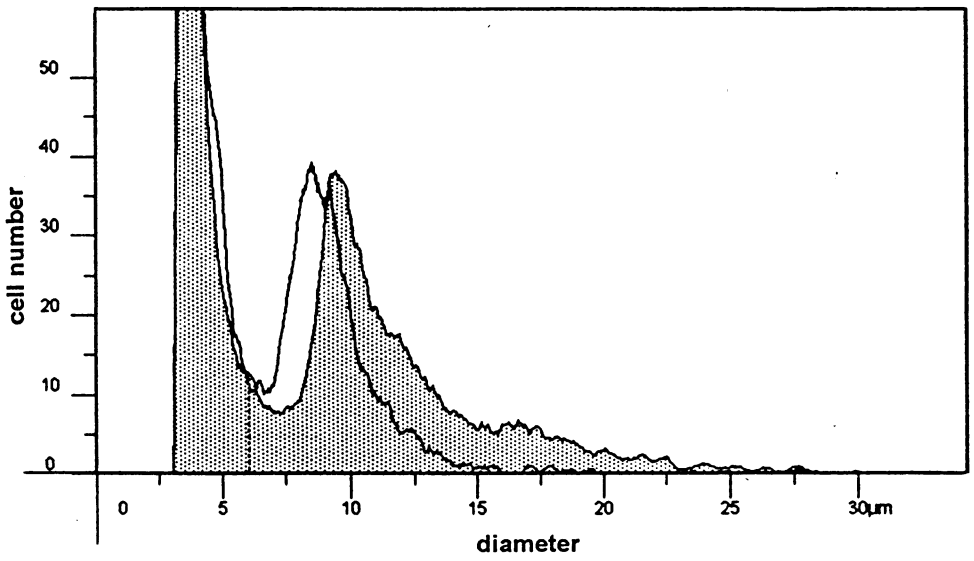

FIG. 5. Size distribution curve of human skin keratinocytes isolated from trypsin-treated epidermal sheets (following thermolysin treatment). After 2 min the small basal keratinocytes with a mean diameter of about 8 µm (open curve) predominate. After 15 min the bigger differentiated keratinocytes with a mean diameter of about 10 µm (stippled curve) represent the vast majority of cells.

ladder formation shown in 1000 and 100 cell equivalents. Additionally, telomerase activity was found in keratinocytes isolated from donors of different ages (ranging from 17 to 90 years), suggesting that the enzyme activity is not donor age-dependent.

Recently, it has been reported that normal human skin contained no telomerase activity (15). Therefore, to exclude the possibility that the telomerase activity in our cultured keratinocytes was an artifact caused by culture conditions, we analyzed several specimens of human skin and foreskin in more detail. In four of nine samples of complete skin, consisting of the epidermis and the underlying dermis (Fig. 4*A*), we detected telomerase activity. A faint ladder formation was seen with protein concentrations of 6 µg (Fig. 4*D*, lane 1) but not with 0.6 or 0.06 µg (Fig. 4*D*, lanes 2 and 3). This applied to trunk skin as well as foreskin samples.

Because isolated fibroblasts were telomerase-deficient and keratinocytes exhibited enzyme activity it was suggested that telomerase present in the skin derived from the epidermis. To test this, we separated the epidermis from the dermis by thermolysin treatment. Under these conditions, the basement membrane stays with the intact dermis (Fig. 4*B*). Similarly, the epidermis remains intact with the proliferative basal layer, the differentiated suprabasal layers, and the dead stratum corneum (Fig. 4*C*). As expected, the dermal compartment was always telomerase deficient (Fig. 4*D*, lanes 4–6). However, extracts from the epidermis reproducibly exhibited telomerase activity in samples consisting of 6, 0.6, and often 0.06 µg of total protein (Fig. 4*D*, lanes 7–9).

Furthermore, to define which epidermal cell layer expressed enzyme activity, the thermolysin isolated epidermal sheets were subjected to additional trypsin treatments. Due to the impermeability of the stratum corneum, trypsin penetrates into the epidermis through the basal layer (and to a small extent through the sides of the sheets). Thus, the cells are released from the basal, spinous, and granular layers in a time-dependent manner. After 2 to 5 min in trypsin the mostly small basal cells with a size of about 8 µm were released as single cells, as demonstrated in the size distribution curve (Fig. 5). With increased trypsinization time (≥5 min) more differentiated cells, as well as the basal cells, were detached from the epidermal sheet. With further trypsinization (15 min), the larger differentiated cells predominated the released cells. This was clearly shown in the size distribution pattern with a shift to a mean size of 10 µm (Fig. 5).

The origin of the different cell types was further verified by immunohistochemistry using antibodies against epidermis-specific keratins. This is feasible because proliferative basal keratinocytes express different keratins than do differentiated keratinocytes. Following various times of trypsinization the remaining epidermal sheets were subjected to immunohistochemistry to determine the presence of basal and differentiated cells by double-labeling with antibodies staining either the complete epidermis or only the differentiated keratinocytes. After 2 min of trypsin treatment all basal cells were released from the epidermal sheet leaving behind cells that only stained positive for the differentiation-specific keratins (Fig. 6*A*). After 5 min some differentiated cells were released (in addition to the basal cells) (Fig. 6*B*) and finally after 15 min all vital cells were disaggregated and only the dead stratum corneum remained intact (Fig. 6*C*).

Telomerase activity was found in lysates of the 2-min "basal cell" fractions in 1000 and 100 cell equivalents (Fig. 6*D*). After 5 min of trypsin treatment and correlating with an increasing number of differentiated cells telomerase activity could only be detected in the 1000 cell equivalent (Fig. 6*D*). In cell lysates from fractions where all epidermal cells were detached from the epidermal sheets (≥15 min of trypsin treatment), telomerase activity could no longer be detected (Fig. 6*D*). To exclude that loss of telomerase activity in this fraction might be due to prolonged trypsinization, cultured keratinocytes and HaCaT cells were subjected to trypsin treatment for up to 25 min prior to the TRAP assay. Telomerase activity in these cells remained unchanged (data not shown), demonstrating that telomerase was not inhibited by long-term trypsin treatment.

As a further control we investigated whether telomerase deficiency may have been due to cell or tissue derived telomerase inhibitors. An equivalent of 100 HaCaT cells was mixed with either a 1000 cell equivalent of keratinocytes (derived from the epidermis after 15 min of trypsin treatment), a 1000 cell equivalent of cultured fibroblasts, or 6 µg of dermal extract. No change in telomerase activity of the HaCaT cells could be detected in the first two mixtures, but a decrease in intensity was observed in the mixture containing dermal extract (data not shown). Thus, telomerase inhibitors may exist in the dermis. However, in mixtures with the 15-min keratinocyte fraction, HaCaT cells maintained their full telomerase

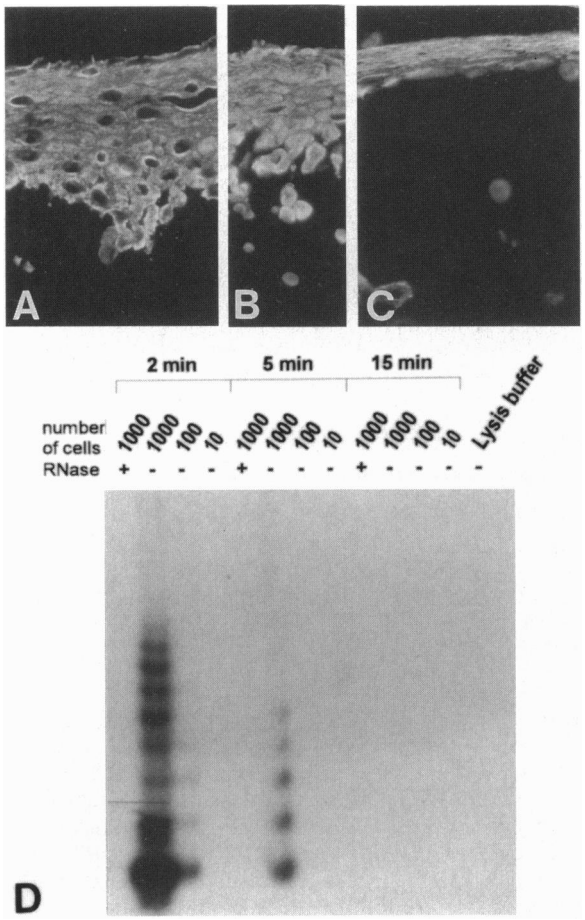

FIG. 6. Identification of the telomerase-positive cells in the epidermis. (*A–C*) Treatment of the thermolysin-isolated epidermis with trypsin solution and immunofluorescence of the remaining epidermal sheet labeled with an antibody against the differentiation specific keratins K1 and K10. Due to the impermeability of the stratum corneum trypsin predominantly penetrates into the epidermis through the basal layer and depending on the incubation time releases different fractions of keratinocytes. (*A*) After 2 min of trypsin treatment mostly basal cells were released from the epidermal sheet leaving behind all differentiated layers. (*B*) After 5 min, in addition to the basal cells, some differentiated keratinocytes also detached. (*C*) After 15 min of trypsin treatment all vital cells were released and only the dead stratum corneum was left. (*D*) Telomerase activity was measured from the same cell fractions (2, 5, and 15 min after trypsin treatment) containing the basal keratinocytes and different amounts of differentiated keratinocytes. The highest telomerase expression was found in the basal cell-enriched fraction. With prolonged time in trypsin (5 min), which released additional differentiated keratinocytes, telomerase activity decreased. After long-term trypsin treatment (≥15 min), when all vital cells were released from the stratum corneum and the basal keratinocytes only represented a minor fraction, telomerase activity was below detection level.

activity suggesting that no inhibitors were present in the keratinocytes but that telomerase activity was reduced by telomerase-negative cells.

DISCUSSION

In this study we investigated human skin keratinocytes, which either gained permanent growth by three different modes of *in vitro* immortalization—SV40, HPV 16, or spontaneously—or were established from human skin SCCs. In each case telomerase activity was detected in as few as 10 cell equivalents, which is in agreement with previous reports noting the correlation of telomerase activity with immortalization (3, 4). Furthermore, we show that in our spontaneously immortalized HaCaT cells, telomerase activity was present in its full expression pattern from the earliest passage (p2) tested. In addition, all cells of this early passage exhibited the same cytogenetic changes (18), which revealed their clonal origin. Because these cytogenetic changes were likely to be involved in the immortalization process of the HaCaT cells, immortalization probably occurred in a single precursor HaCaT cell in the primary culture, allowing that particular cell to expand progressively while the other cells died. These data suggest that full expression of telomerase activity correlated with a very early stage in this immortalization process and that it may represent a useful marker for the switch to immortality. In contrast, Shay and coworkers (28) reported that under normal culture conditions most SV40 T-antigen transfected human fibroblasts failed to immortalize. Only after a crisis period did a few fibroblast cell clones emerge that could be established as immortal cell lines. In these fibroblast cultures telomerase activity increased as the passage number increased (28) and at present, it cannot be excluded that the discrepancy in the telomerase expression patterns may be cell type specific.

Telomerase activation is probably not the only mechanism by which telomere length can be maintained. Recently 15 of 35 DNA tumor virus-transformed fibroblast lines were described. They all had long and heterogeneous telomeres, but lacked telomerase activity (29), suggesting that an alternative regulation of telomere length may exist. However, the precise method of regulation has yet to be determined.

In addition to telomerase activity in immortal and tumor-derived human keratinocytes, we also showed telomerase activity in 4 of 9 human skin samples and in 14 different cultures of normal human skin keratinocytes. These findings were unexpected since in an earlier report skin was described to be telomerase deficient (15) and Lindsey and coworkers showed loss of telomeric repeats *in vivo* with age in humans (30). However, this discrepancy can be explained by the difference in using total skin versus separated epidermis. In our hands, total human skin was telomerase deficient 55% of the time. Only after separating the epidermis from the dermis did we find reproducible telomerase activity.

The epidermis, unlike the dermis, is a continually renewing tissue in which basal cells divide to replace squames that are sloughed into the environment. The telomerase findings in this study correlate with the fact that keratinocytes in the epidermis must continue to proliferate throughout the lifetime of the tissue to replenish the continually sloughed cells. Any premature loss of proliferative capacity in the epidermis would be fatal. Thus, the basic telomerase activity detected in our specimens might be expected to be found in the epidermis and might be involved in maintaining this tissue's regenerative potential. On the other hand, fibroblasts of the dermis are generally arrested in a nonproliferative stage and are only stimulated to proliferate under pathological conditions, e.g., during wound healing. It is tempting to speculate that in tissues in which proliferation is generally absent, telomerase activity is not required to maintain tissue integrity.

Our findings and interpretations are also supported by recent reports. Despite declining telomeres, telomerase was found to be expressed in primary human hematopoietic cells (12–14). In addition, we have preliminary evidence that the epithelium of the intestine is telomerase positive (C.H.-B. and P.B., unpublished observations). On the other hand, human normal mucosa, a tissue structurally very similar to the epidermis, has been reported as telomerase negative (31). However, it is not clear whether the epithelium lacks telomerase or whether the absence of enzyme activity is due to a dilution effect by the telomerase negative stroma.

Our cell fraction experiments indicated that in the epidermis enzyme activity is restricted to the basal layer, with good activity seen only in the populations consisting predominantly of proliferative basal cells. When differentiated suprabasal

cells were present, the signal was diluted below the level of detection. Due to the scarcity of tools available to detect telomerase activity *in situ* and to distinguish functionally different cell types in the basal layer of the epidermis, we were not able to determine whether all basal cells or only a subfraction, e.g., the putative stem cells, express telomerase activity. If all basal cells express telomerase, then the enzyme activity must be upregulated in immortal and tumor cells because telomerase activity is generally increased in these cells (2–6), as it was in our keratinocyte cell lines. On the other hand, if telomerase activity is restricted to only the stem cells with the other basal cells showing no activity, then activity in the stem cells must be very strong because it is easily detected in the cell fraction that contains the whole basal cell population. If the latter hypothesis is correct, then stem cells might be precursors of immortal and tumor cells. It has been previously postulated that stem cells proliferate only sporadically, are maintained in the basal layer of the epidermis, and are optimal targets for transformation (32). After clonal expansion, their daughter cells would then maintain the "high" level of telomerase expression. However, the presence of telomerase in epidermal stem cells remains to be determined.

Our data indicate that telomerase activity is important for long-life *in vivo* and permanent growth *in vitro*. In addition, our data suggest an alternative to the general interpretation that telomerase must be reactivated in immortal and tumor cells. For normal tissues, such as the epidermis, which exhibit telomerase activity, the telomerase positive cells might be the precursors of immortal and tumor cells, and instead of reactivation of telomerase activity, positive cells would be selectively expanded.

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